Microbial biofilm formation on soybean oil based polymers

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Abstract

In this study, biofilm formation on different type of epoxidized soybean oil (ESO) based polymers was investigated. Different types of ESO based polymers were synthesized. Bacterial accumulations on surface of these polymers were determined by using Modified Robbins Device for 60 days period. Surface characterizations of the materials were also performed at the beginning, at the 30th day and the 60th day of the experiment. Except ESO-HMDA polymer, changes were observed on the other polymer surfaces. Among the polymers synthesized, bacterial accumulation was the highest on the surfaces of the ESO-PEGM and ESO-TETA polymers. On the other hand, biofilm formation on the ESO-BPA and AESO-acrylic acid copolymer was the lowest. After 60 days, however, bacterial accumulation was highest on the surfaces of the ESO-TETA, ESO-MA and ESO-BPA polymers. Changes of the functional groups on the surface of the polymers were followed by MIR spectroscopy. It was found that almost all the surfaces of the materials were changed by microorganisms. When the surface was invaded by bacteria, microorganisms began to consume triglyceride moiety first.

Keywords: Soybean oil, polymers, biofilms, Modified Robbins Device
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Soya Yağı Bazlı Polimerlerde Mikrobiyal Biyofilm Oluşumu

Özet

Bu çalışmada epoksi soya yağı (ESO) bazlı farklı türde polimerlerin biyofilm oluşumu potansiyelleri incelenmiştir. ESO bazlı polimerlerin farklı türleri sentezlenerek bu polimerlerin yüzeyinde 60 gün süreyle Modifiye Robbins Cihazı kullanılarak bakteriyel biyofilmlerin oluşumuna imkan tanınmıştır. Malzemelerin yüzey karakterizasyonu deney öncesinde, 30. ve 60. günlerde yapılmıştır. ESO-HMDA polimeri dışında, diğer polimer yüzeylerinde önemli değişimler gözlenmiştir. Polimerler arasında yoğun biyofilm oluşumu 30. günde ESO-TETA polimer yüzeylerinde gözlenirken en düşük değerler ESO-BPA ve AESO-akrilik asit kopolimer üzerinde tespit edilmiştir. Altmış gün sonunda, en yüksek mikrobiyal biyofilm birikimi ESO-TETA, ESO-MA ve ESO-BPA polimer yüzeylerinde tespit edilmiştir. Çalışmada kullanılan polimerlerin yüzeyindeki fonksiyonel grupların değişiklikler MIR spektroskopisi ile izlenmiştir. Bu malzemelerin hemen hemen tüm yüzeyleri mikroorganizmalar tarafından değiştirilmiş olduğu tespit edilmiştir. Yüzey bakteriler tarafından kolonize edildiğinde, mikroorganizmaların ilk olarak trigliserit bakiyelerini tükettiği gözlenmiştir.

Anahtar Kelimeler: Soya yağı, polimer, biyofilm, Modifiye Robbins Cihazı

Introduction

Non-petroleum based polymers are attracting more attention nowadays. Especially fatty based polymers attracted more attention because one can easily synthesize a wide variety of soft, strong on load bearing polymers by using fine chemicals which are synthesized from plant oil triglycerides (Cayli, 2008). Environmental concerns are another factor. In the case of environmental sense, having biodegradable properties is vital. There are lots of works reported in the literature about the environmental impacts and biodegradability of the triglyceride based polymers (Wool, R. 2005). These works are mainly focus on the decomposition of the oil based polymers in soil (Shogren, 2004). During tests, polymer specimens are buried in soil and some parameters such as the released amount of the CO_2, weight lost of the samples are measured (Doğan, 2008).

Seventy per cent of the world is water and burying of the polymeric materials in water is also an important issue. When a material is contacted with water, the surface of it is invaded by microorganisms. Then these microorganisms form a biofilm. Biofilms are composed of populations or communities of microorganisms adhering to variety of surfaces. These microorganisms are usually encased in an extracellular polysaccharide that they themselves synthesize. Biofilms can contain many different types of microorganism, e.g. bacteria, archaea, protozoa, fungi and algae; each group performing specialized metabolic functions (Costerton, 2007). In general, the formation of bacterial biofilms is believed to take place over at least three stages: a reversible adsorption step, primary adhesion of microorganisms to a surface, and colonization. The rates of these processes vary widely depending on the environmental conditions and the type of microorganisms, but the adhesion and colonization stages are considered to be relatively slow compared to the first step of cell adsorption. In principle, it should be possible to retard, if not prevent, the formation of biofilms on substrates by using materials to which bacteria cannot initially attach, and such a material or surface coating would be of considerable commercial interest. In practice, however, synthetic materials that are capable of preventing bacterial adsorption have proved rather elusive, despite a significant volume of research

Properties of the substrate, such as hydrophobicity, hydrophilicity, steric hindrance, roughness, and the existence of a "conditioning layer" at the surface, are all thought to be important in the initial cell attachment process. The prevention of contamination caused by pathogenic microorganisms during the manufacture, processing, and packaging of food is of considerable importance to public health and consequently is a major issue for industry (Characklis, 1981).

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Properties of the substrate, such as hydrophobicity (Schackenraad, 1992), hydrophilicity (Kiss, 1996), steric hindrance (Kuhl, 1994), roughness (Kiaie, 1995), and the existence of a "conditioning layer" at the surface (Abarzua, 1995), are all thought to be important in the initial cell attachment process.

The prevention of contamination caused by pathogenic microorganisms during the manufacture, processing, and packaging of food is of considerable importance to public health and consequently is a major issue for industry (Morton, 1994).

According to the best of our knowledge there is no reported literature found for the biofilm formation on the triglyceride based polymers. Therefore, the adhesion affinities of different candidate materials were tested regarding bacterial accumulation using Modified Robbins Device (MRD). Fatty based polymers that are produced from different functional monomers were compared against changes on their surfaces by the aid of Multiple Internal Reflectance (MIR) spectroscopy before and after the experimental procedure.

Materials and methods

The biofilm was established under standard hydraulic conditions and tubular pipe flow using a MRD, which yields a non-turbulent laminar flow (Reynolds number 0.6). The Robbins device (McCoy, 1994) is a flow system in which are inserted several removable plugs on which biofilm forms. The Reynolds number is used to describe the turbulence. Discs of different material were immobilized on MRD plugs and the experimental setup was run 60-days period. The device is connected to the distributed drinking water network and was fed continuously. Twelve different materials which were acrylated-ESO [epoxidized soybean oil], acrylated-ESO co-acrylic acid, ESO-phtalic anhydride, ESO-triethylenetetraamine, ESO-hekzamethylenediamine, ESO-bis-phenol A, polyvinylchloride, polyurethane, stainless steel were tested in this study. The bacterial numbers were determined by heterotrophic plate counting (HPC) after 30- and 60-days period. IR characterization of the materials synthesized was performed with Perkin-Elmer 1600 FTIR. The surfaces of polymeric materials were analyzed by MIR spectroscopy to determine whether the chemical modifications were present or not.

Epoxidized soybean oil (ESO) was purchased from the C.P. Hall Company (Memphis –TN). Acrylated soybean oil (AESO) was synthesized in laboratory. Acrylic acid, Malonic acid, Maleic anhydride, 1,4 diazo [222] bicyclo octane (DABCO) was purchased from Merck (Schuchardt-Munich). Bisphenol A was purchased from Aldrich (Milwaukie). PEG 400 was purchased from Fischer Scientific (New Jersey). Heksamethylene diamine (HMDA) and Triethylene tetraamine (TETA) was purchased from Aldrich (Milwaukee). Phthalic acid was purchased from Griffin & George Sales Limited (London, Birmingham). THF (tetra hydro furan) was purchased from J.T. Baker (Deventer-Holland).

Synthesis of ESO-BPA polymer

6 g ESO (6.3 mmol) was mixed with 2.88 g BPA and 0.01 g (0.089 mmol) DABCO 90 °C. Then the mixture was heated at 135°C until DABCO melted. The mixture was then heated for six hours at 150 °C and finally post cured at 200 °C for at least four hours. The product was very rigid solid with transparent brown color.

ESO-HMDA polymer

6 g (6.3 mmol) ESO and 1.57g (13 mmol) HMDA were mixed with 0.01 g (0,089 mmol) DABCO. The mixture was heated to 90 °C then at 100 °C for four hours. Then mixture was post heated at 150 °C for another four hours. The product was light orange colored transparent soft solid.

ESO-TETA polymer

6 g (6.3 mmol) ESO and 0.92 g TETA was mixed with 0.01 g (0.089 mmol) DABCO. Then this mixture was heated to 90 °C then to 100 °C for four hours. Then this pre-cured polymer was post heated to 150 °C for another four hours. The product was orange colored transparent soft solid.

ESO phthalic acid polymer

10 g (0.01 mol) ESO was mixed with 6.8 g (0.044 mol) phthalic anhydride in dry THF (tetra hydro furan) and refluxed for 2 hours. Then THF evaporated and pre polymer was cured at 80 °C for 4 hours. Finally transparent, slightly yellow, and flexible polymer was obtained.

AESO polymer

10 g of AESO was mixed with 0.1 g of AIBN and cured under N_2 at 65 ^oC for 6 hour. Semi transparent hard polymer was obtained.

AESO co acrylic acid polymer

5 g of AESO was dissolved in 5 g of acrylic acid. This solution was mixed with 0.1 g of AIBN and cured under N_2 at 65 ^oC for 6 hour. Transparent and very hard solid was obtained.

Result and discussions

Highest bacterial accumulation was observed on ESO-triethylenetetraamine after 30d period. It is thought that amine moieties on the surface, which caused (+) charge on the surface, attracted more bacteria than carboxyl group modified one. We also observed the relation of bacterial accumulation on the surface with increasing surface charge (Fig. 1, see ESO-hekzamethylenediamine and ESO-triethylenetetraamine). Lowest bacterial accumulation was observed on carboxyl (Fig. 1, see acrylated ESO co-acrylic acid, ESO-phtalic anhydride and ESO-malonic acid) modified surfaces. Bacterial accumulation on neutral surface was higher than acid functionalized one and closer to amine modified ones than acid modified one. Phenolic resin showed the lowest bacterial accumulation first 30 days probably due to antimicrobial effects of bis- phenol A. We also investigated some changes of the functional groups on the surfaces (Fig. 2, 3, 4, 5). Significantly, high count of bacterial accumulation was found on ESO-TETA (Fig. 1). On acrylated ESO co-acrylic acid and bis-phenol, significant differences were observed between 30- and 60-d periods regarding bacterial counts. No significant fluctuations of bacterial counts were found between two periods on ESO-malonic acid, ESO-hekzamethylenediamine, PVC and stainless steel surfaces (Fig. 1). The Robbins device has been used widely in biofilm research, where it is a good model of a class of biofilm problems which arise in water and other liquid distribution systems. Flow rate can easily be varied, allowing for experiments on the effects of shear on biofilm formation.

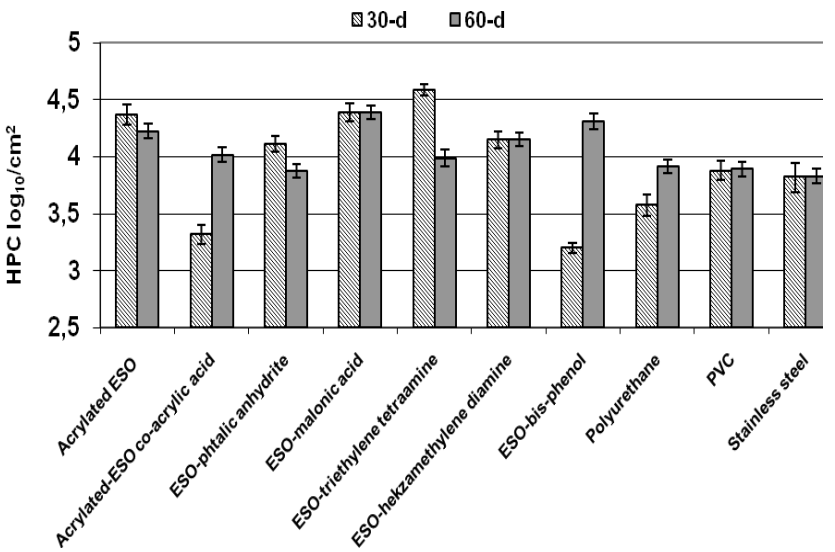

Figure 1. Bacterial counts (HPC) on surfaces after 30- and 60-day periods.

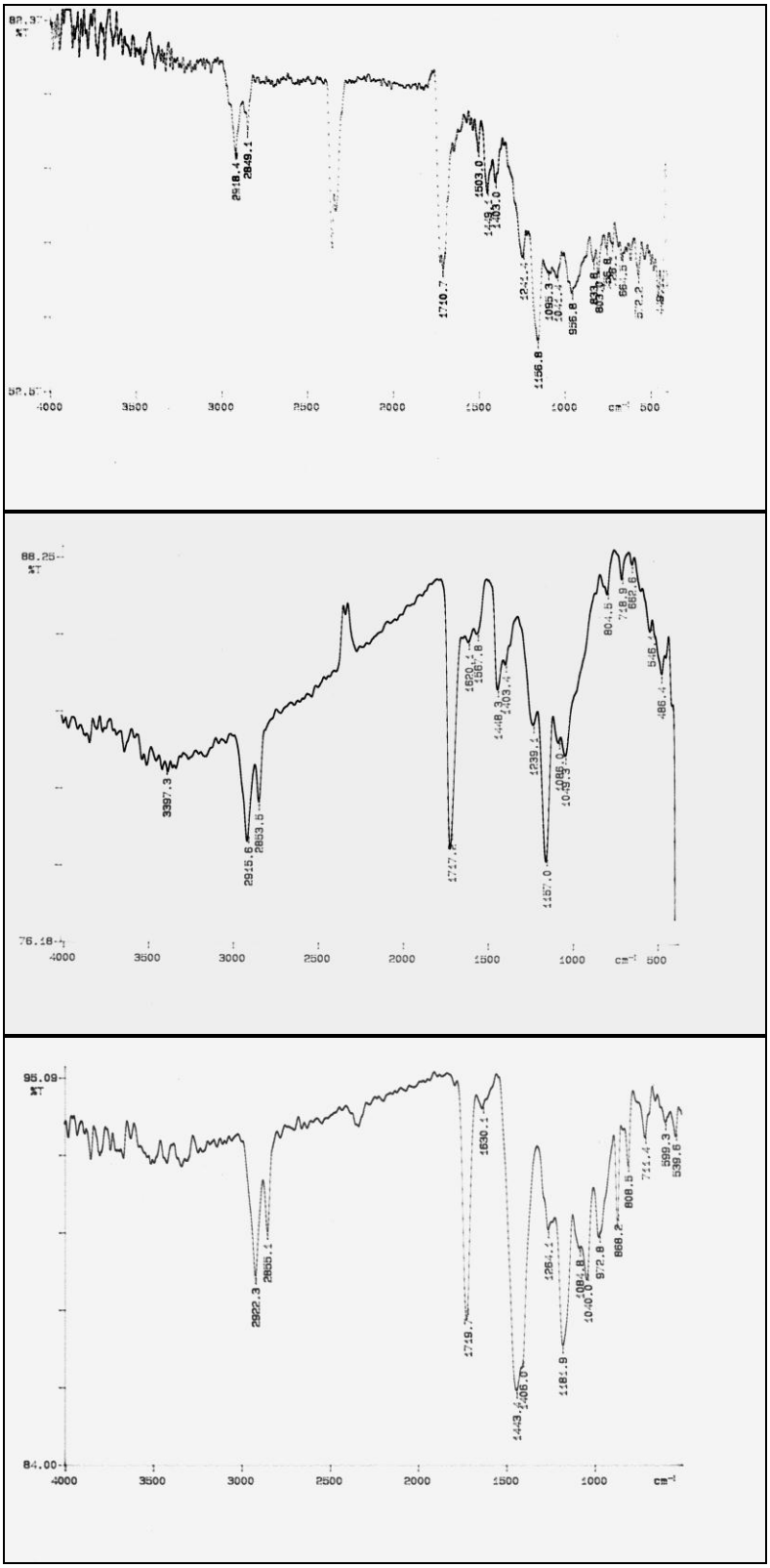

Figure 2. The MIR results of acrylated-ESO co-acrylic acid on day 0, 30 and 60, respectively.

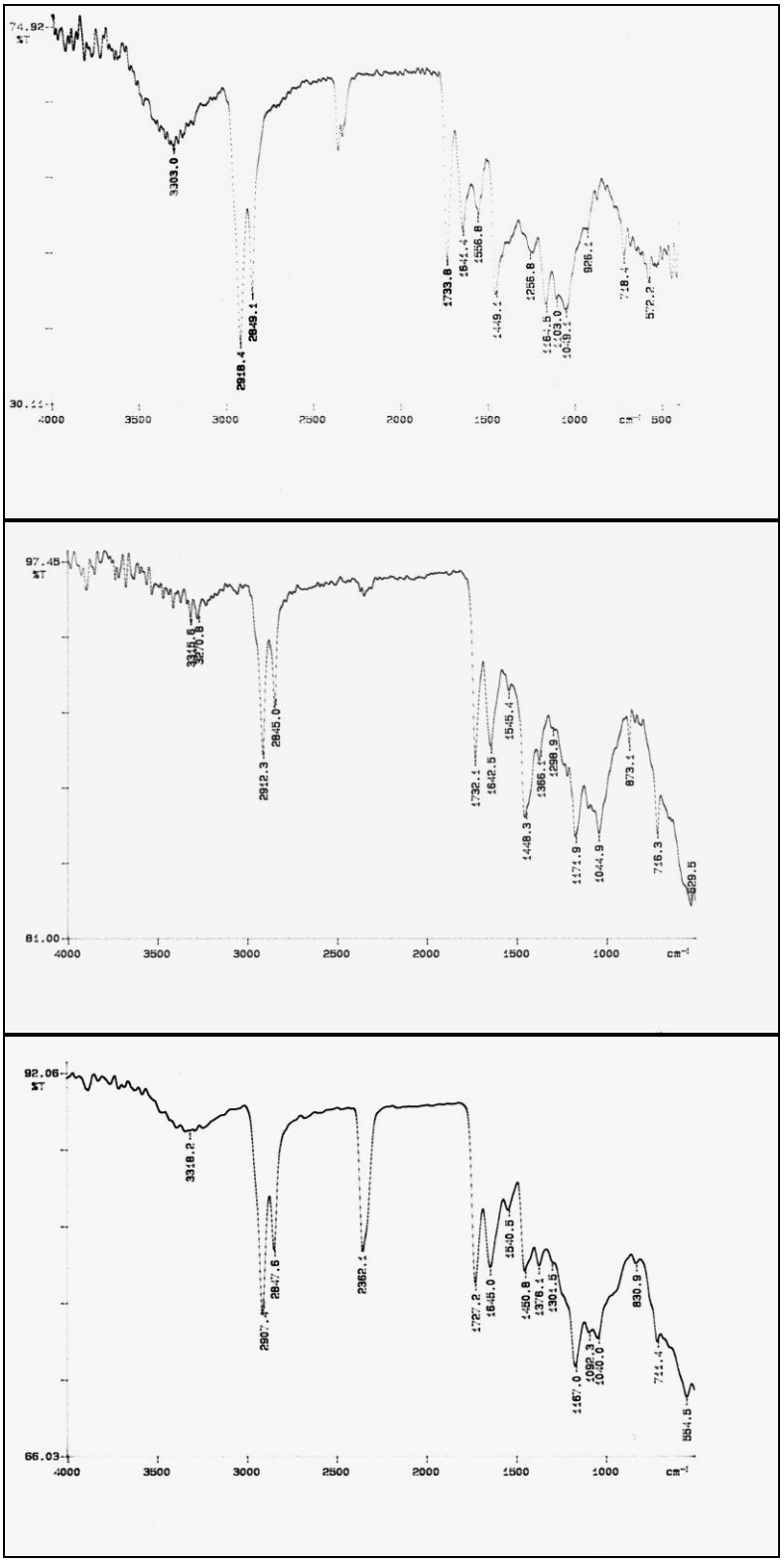

Figure 3. The MIR results of ESO-triethylenetetraamine on day 0, 30 and 60, respectively.

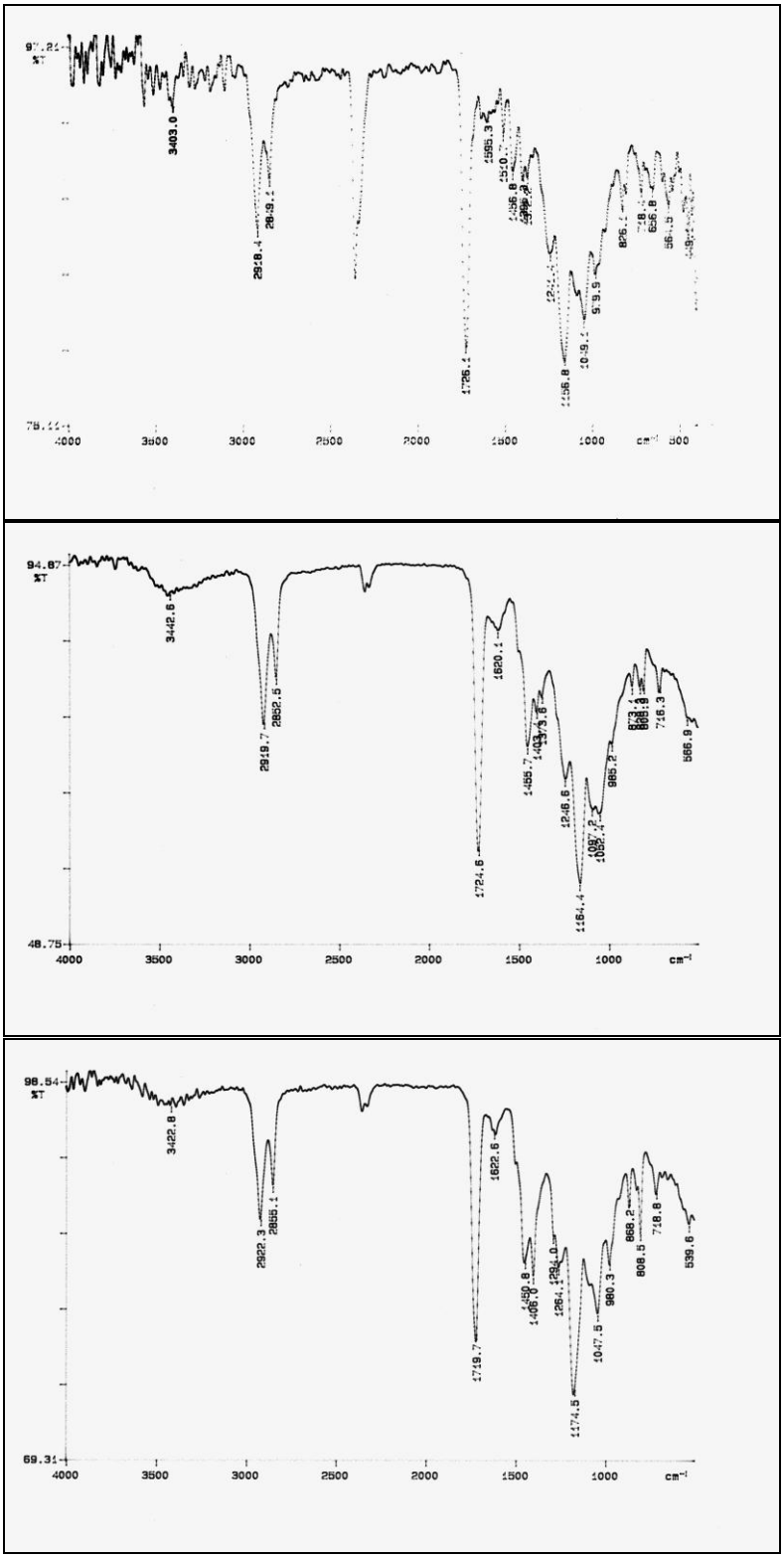

Figure 4. The MIR results of acrylated-ESO on day 0, 30 and 60, respectively.

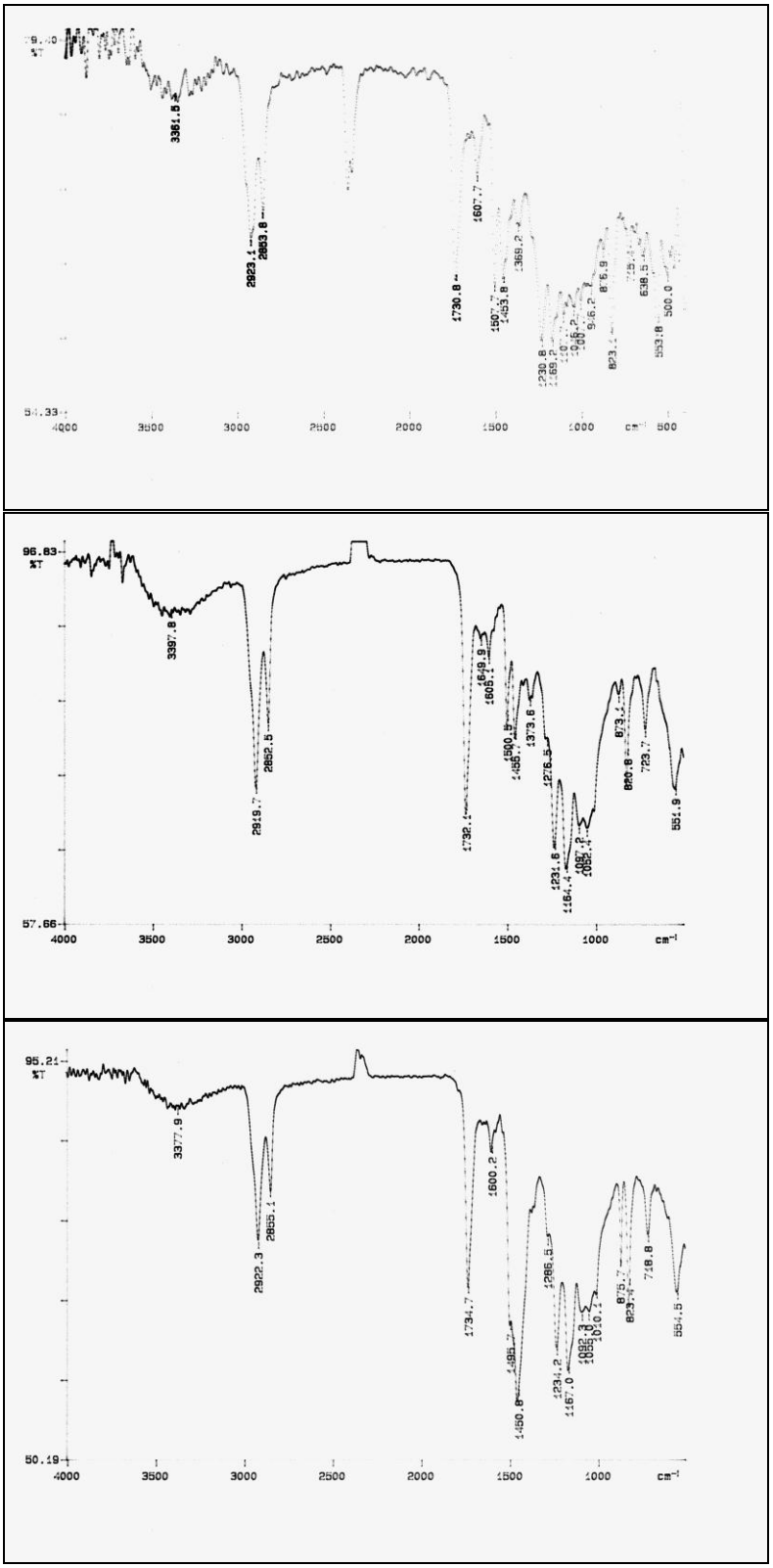

Figure 5. The MIR results of ESO-bis-phenol A on day 0, 30 and 60, respectively.

Conclusions

The amount of bacterial accumulation on the surfaces depends on the functional groups and hydrophobicity of the surfaces. Bacterial accumulation was higher on the amine-functionalized surface than neutral surfaces. The positive charge, which causes from the ionization of amine groups, on the surfaces would attract negatively charged bacteria. When bacteria invade the surface they would digest the fatty parts of polymers. This phenomenon was easily detected by MIR. We could follow the decrease the peaks at around 1741 cm^{-1} which shows the ester groups of fatty materials and increase the IR peaks of other functional groups. This theory well suited with decreasing counts of bacteria for 60 days period. During these periods bacteria first digest the triglyceride on the surface. Thus, amine or carboxyl group amount increases during bacterial action. Finally high functionalization causes increasing or decreasing pH of surface or concentration of functional groups itself appearing toxic effects that stopped the bacterial accumulation. On the non-functionalized surfaces, disappearing of fatty materials triggered dropping of the number of bacteria on the surfaces due to the lack of nutrient.

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